Mechanisms for adverse impact of perinatal infections on pregnancy outcomes

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Silver Spring MD
Key Questions/Concepts

- Mechanism of intrauterine inflammation
- Adverse impact of vaginal dysbiosis
- Fetal and neonatal effects of exposure to intrauterine inflammation – Neonatal immune modulation and susceptibility to wheezing disorder
- Life-long consequences of low lung function at birth
Causes of preterm delivery

- Spontaneous preterm labor: 45%
- Maternal or fetal indication: 30%
- Premature preterm rupture of membrane: 25%

Rhesus macaque: Intraamniotic injection of agonists
Similarities in histologic chorioamnionitis
Human vs. Rhesus Macaque

Human

IA LPS injection model
Rhesus Macaque

Amniotic Fluid
Similar cellular composition of the chorionic decidua in Rhesus and humans

Presicce et al. 2018
Animal model

Preterm pregnant Rhesus macaque model of IUI induced by IA LPS

- Similarities between Rhesus and human reproductive systems, Endocrinology, Placenta architecture and immunology

![Diagram showing the animal model with IA LPS injections at -3 h, -1 h, and 0 h, followed by C section at 16 h, and SC and IA injections of rhIL-1ra (Anakinra) at 100mg and 50mg respectively.]

Presicce et al 2018
IL-1ra decreases LPS induced neutrophil accumulation in the chorio-decidua

Presicce et al JCI insight March 2018
IL-1ra decreases LPS induced neutrophil TNF expression

Chorio-decidua neutrophil TNFα (unstimulated)

Presicce et al JCI insight March 2018
Presicce et al. *JCI insight* March 2018

Inflammatory products/microorganisms

Amnion

Chorion

Decidua

pIRAK-1 (amnion) → rhIL-1ra
CSF3, CXCL8
Neutrophil infiltration
rhIL-1ra ↔ Bcl2A1
TNFα
Neutrophil activation & pro-survival program
Macrophages and other cells
Intrauterine inflammation

Presicce et al. *JCI insight* March 2018
Vaginal dysbiosis predisposes to adverse pregnancy outcomes

Prospective Antenatal vaginal microbiota sampling of women in London UK

Correlation with later pregnancy outcomes

Brown et al *BMC Medicine* 16:9:2018
Vaginal dysbiosis predisposes to preterm delivery

Longitudinal weekly vaginal microbiome sampling beginning early pregnancy in 40 women

DiGiulio et al. PNAS 112:11060:2015
Neonatal microbial colonization depends on the mode of delivery: Vaginal vs C-section

Summary - I

• IL1 signaling is important in the pathogenesis of intrauterine inflammation
• Deficiency of Lactobacilli in the vagina predisposes to adverse pregnancy outcomes
• Neonatal colonization is dependent on the mode of delivery
Fetal and Neonatal consequences of Chorioamnionitis?

- Fetal sepsis and intrauterine or immediate postnatal death
- Early onset Neonatal sepsis
- Neonatal morbidities (BPD, Neurodevelopment)
- Asthma, Immune modulation
- No apparent morbidities
Prematurity and Chorio increase risk for childhood asthma

Chorio diagnosed clinically

No chorio

<table>
<thead>
<tr>
<th></th>
<th>6-12 Months of Age</th>
<th>18-24 Months of Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chorio (n=46)</td>
<td>Chorio (n=46)</td>
</tr>
<tr>
<td></td>
<td>No Chorio (n=138)</td>
<td>No Chorio (n=138)</td>
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<tr>
<td></td>
<td>Caregiver report of wheezy symptoms (%)</td>
<td>Caregiver report of wheezy symptoms (%)</td>
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<tr>
<td></td>
<td>Chorio (n=46)</td>
<td>50%</td>
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<tr>
<td></td>
<td>No Chorio (n=138)</td>
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<td></td>
<td>Unadjusted OR (CI)</td>
<td>1.98 (1.00, 3.92)</td>
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<tr>
<td></td>
<td>Adjusted OR (CI)*</td>
<td>2.08 (0.99, 4.40)</td>
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<td></td>
<td>Doctor visit for respiratory problems</td>
<td>Doctor visit for respiratory problems</td>
</tr>
<tr>
<td></td>
<td>Chorio (n=46)</td>
<td>49%</td>
</tr>
<tr>
<td></td>
<td>No Chorio (n=138)</td>
<td>25%</td>
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<tr>
<td></td>
<td>Unadjusted OR (CI)</td>
<td>2.82 (1.39, 5.69)</td>
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<tr>
<td></td>
<td>Adjusted OR (CI)*</td>
<td>3.18 (1.45, 7.00)</td>
</tr>
<tr>
<td></td>
<td>Physician diagnosed bronchitis, bronchiolitis or pneumonia</td>
<td>Physician diagnosed bronchitis, bronchiolitis or pneumonia</td>
</tr>
<tr>
<td></td>
<td>Chorio (n=46)</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>No Chorio (n=138)</td>
<td>16%</td>
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<tr>
<td></td>
<td>Unadjusted OR (CI)</td>
<td>2.31 (1.06, 5.04)</td>
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<td></td>
<td>Adjusted OR (CI)*</td>
<td>2.02 (0.85, 4.8)</td>
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<tr>
<td></td>
<td>Respiratory medication prescription (%)</td>
<td>Respiratory medication prescription (%)</td>
</tr>
<tr>
<td></td>
<td>Chorio (n=46)</td>
<td>35%</td>
</tr>
<tr>
<td></td>
<td>No Chorio (n=138)</td>
<td>14%</td>
</tr>
<tr>
<td></td>
<td>Unadjusted OR (CI)</td>
<td>3.25 (1.02, 10.32)</td>
</tr>
<tr>
<td></td>
<td>Adjusted OR (CI)*</td>
<td>3.72 (0.87, 15.95)</td>
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</tbody>
</table>

Tregs from moderate/late preterm infants have lower T-cell suppressive capacity

The immunosuppressive Tregs from preterm infants are less functional compared to term
Tregs from preterms with chorio are less functional compared to preterms without chorio

Higher cord IL-6 levels is associated with adverse pulmonary outcomes at 6-12 months in moderate/late preterm infants

Cincinnati Chorio study

<table>
<thead>
<tr>
<th></th>
<th>Cord blood IL-6 &lt;11 pg/mL (n=109)</th>
<th>Cord blood IL-6 ≥11 pg/mL (n=25)</th>
<th>OR (CI) for high vs. low IL-6</th>
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</thead>
<tbody>
<tr>
<td>Caregiver report of</td>
<td>33%</td>
<td>52%</td>
<td>2.17 (0.89, 5.27)</td>
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<tr>
<td>wheezy symptoms at 6-12</td>
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<tr>
<td>months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doctor visit for respiratory</td>
<td>27%</td>
<td>36%</td>
<td>1.53 (0.60, 3.88)</td>
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<tr>
<td>problems at 6-12 months</td>
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<td>Emergency room visit for</td>
<td>50%</td>
<td>33%</td>
<td>0.50 (0.10, 2.42)</td>
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<tr>
<td>respiratory problems at 6-12</td>
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<td></td>
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<tr>
<td>months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physician diagnosed</td>
<td>15%</td>
<td>36%</td>
<td>3.23 (1.21, 8.64)*</td>
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<tr>
<td>bronchitis, bronchiolitis or</td>
<td></td>
<td></td>
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<tr>
<td>pneumonia at 6-12 months</td>
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</tbody>
</table>

Intrauterine inflammation

During infancy

Poor lung function
Low lung function in early infancy predicts low lung function at adulthood

Quartiles of $V'_{\text{max}}_{FRC}$ at $\leq 3$ months (Maximal expiratory flows at FRC in 123 Full term healthy infants)

Summary

• In late-preterm Cincinnati chorio study, chorioamnionitis did not significantly increase early NICU morbidity

• Chorio exposure and increased cord blood IL-6 increases risk for wheezing for up to 2 years

• Low lung function at birth has life-long consequences
Questions

1) Can intrauterine infection/inflammation be prevented or treated?
2) Can restoration of normal vaginal microbiota decrease adverse pregnancy outcomes?
3) Can vaginal probiotic therapy decrease risk for intrauterine inflammation induced abnormal infant Treg function and decrease risk for wheezing disorders in infancy?
Acknowledgements

**Kallapur Lab**
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**U.C. Davis CA**
- Lisa Miller

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- March of Dimes (Kallapur)
- Perinatal Institute Cincinnati – ARC (Chougnet)
Possible mechanisms of benefits of Lactobacilli probiotics – Microbiota restoration

- Co-aggregation
- Bio-surfactant production
- Bacteriocin and \( \text{H}_2\text{O}_2 \) production
- Competitive exclusion
- Immune modulation
- Modulation of tight junctions
- Signaling effects

References


AF infection and severe inflammation is associated with preterm delivery


% remaining undelivered

Latency (days)

Organisms        AF IL-6 ng/mL

- Infection       + > 11.3
- Severe Inflammation - > 11.3
- Mild Inflammation  - 2.6-11.3
- Colonization     + < 2.6
- Negative AF      - < 2.6

Multi-center predictive biomarker study – US
305 Women with spontaneous PTL with intact membranes

FACS Gating strategy for Immuno-phenotyping Rhesus Chorio-Decidua Cells

SUMMARY - I

• Intrauterine inflammation – Histologic correlate is chorioamnionitis
• Neutrophil infiltration of the choriodecidua
• Maternal-fetal interface has many different leukocytes
• Rhesus macaque models are similar to human pathology
IL-1ra decreases LPS induced inflammation in the AF

Presicce et al 2018
IL-1ra decreases LPS induced neutrophil IL8 expression

Presicce et al 2018
• IL-ra decreased neutrophil accumulation and activation in the chorio-decidua
• mRNA seq of chorio-decidua cells revealed that Bcl2A1 or Bfl1 is a highly induced gene both in the Rhesus macaque and humans
Intirinsic pathway of Apoptosis
IL-1ra decreases LPS induced neutrophil Bcl2A1 expression
What signals might mediate neutrophil migration to the chorio-decidua?
IL-1ra decreases LPS induced Amnion p-IRAK1 expression

$\text{IL-8/CXCL8}$  $\text{CSF3}$  $\text{IL-1\beta}$

mRNA fold change

Presicce et al 2018
AF inflammation is not invariably associated with preterm delivery.

Multi-center predictive biomarker study – US
305 Women with spontaneous PTL with intact membranes

AF IL6 2.6 -11.3 ng/mL

IFNAR signaling is necessary for infection-mediated priming of LPS-driven PTB

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Compartment</th>
<th>Dose</th>
<th>Duration</th>
<th>PTL</th>
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<tbody>
<tr>
<td>IL-1β</td>
<td>IA</td>
<td>1 µg</td>
<td>3d</td>
<td>0/4</td>
</tr>
<tr>
<td>IL-1β</td>
<td>IA</td>
<td>1 µg</td>
<td>1d</td>
<td>0/9</td>
</tr>
<tr>
<td>LPS (E. coli)</td>
<td>IA</td>
<td>1 mg</td>
<td>2d</td>
<td>0/8</td>
</tr>
<tr>
<td>LPS (E. coli)</td>
<td>IA</td>
<td>1 mg</td>
<td>5d</td>
<td>0/9</td>
</tr>
<tr>
<td>Live U. Parvum</td>
<td>IA</td>
<td>$1 \times 10^7$ CFU</td>
<td>3d or 7d</td>
<td>0/10</td>
</tr>
<tr>
<td>Live E. coli</td>
<td>IA</td>
<td>$1 \times 10^6$ CFU</td>
<td>2d</td>
<td>5/5**</td>
</tr>
<tr>
<td>Live E. coli + Abx*</td>
<td>IA</td>
<td>$1 \times 10^6$ CFU</td>
<td>3d</td>
<td>6/8**</td>
</tr>
<tr>
<td>Live E. coli</td>
<td>Chorio-decidua</td>
<td>$7 \times 10^6$ CFU over 72h</td>
<td>3d</td>
<td>0/2</td>
</tr>
</tbody>
</table>

**P<0.01 vs. IA LPS. IA = intraamniotic

*Antibiotics – given IM + IA starting 24h after IA E. coli.
Chorioamnion-decidua
Highly upregulated genes (>50 fold)

Moderately upregulated genes (<50 fold)

\[ * = p<0.05 \text{ compared to control} \]

Values normalized to control group

Kannan, Cappellitti et al 2018
Summary

- E. coli infections induce rapid PTL in Rhesus
- Intraamniotic invasion appears to increase the risk for PTL
- IL6 and CCL2 expression in the choriodecidua correlates with PTL
- IFN/IFNAR axis may be an important modulator of risk for PTL
Tregs from moderate/late preterm infants have lower T-cell suppressive capacity

The immunosuppressive Tregs from preterm infants are less functional compared to term Tregs from preterms with chorio are less functional compared to preterms without chorio

iPFT technique

N=70 late preterm infants

Testing was performed on the nSpire Infant Pulmonary Lab system (Longmont, CO) using the raised-volume rapid thoracoabdominal compression technique and infant plethysmography according to the American Thoracic Society/European Respiratory Society guidelines.
Infant PFT at 6-12 months in a cohort of moderate/later preterm infants

McDowell et al
Multivariable Analysis of Association of Race and Gender with abnormal PFT

<table>
<thead>
<tr>
<th></th>
<th>FEV (_{0.5}) % Pred</th>
<th>FVC % Pred</th>
<th>FEV/FVC % Pred</th>
<th>FEF75 % Pred</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Black</td>
<td>-15.78 (-26.61, -4.96)</td>
<td>-14.10 (-25.57, -2.62)</td>
<td>-1.11 (-7.10, 4.87)</td>
<td>-19.07 (-40.80, 2.66)</td>
</tr>
<tr>
<td>Mixed</td>
<td>-7.21 (-21.81, 7.40)</td>
<td>-10.95 (-26.43, 4.52)</td>
<td>3.95 (-4.12, 12.02)</td>
<td>0.35 (-28.97, 29.66)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Female</td>
<td>9.22 (0.59, 17.84)</td>
<td>5.15 (-3.99, 14.29)</td>
<td>4.66 (-0.11, 9.42)</td>
<td>26.47 (9.16, 43.79)</td>
</tr>
<tr>
<td>Chorio</td>
<td>-1.42 (0.78, -11.54)</td>
<td>1.21 (-9.52, 11.93)</td>
<td>-2.20 (-7.79, 3.40)</td>
<td>-8.60 (-28.92, 11.71)</td>
</tr>
</tbody>
</table>

- Model Adjusted for Gender, Race, Gestational Age, Insurance status, Chorio, Use of Ventilation and Smoking Exposure

Venkatesh, Goyal et al (2016)
Pulmonary outcomes at 6-12 months based on severity of chorioamnionitis in moderate/late preterm infants

Incidence of histologic chorioamnionitis was 20%

Distribution of Chorioamnionitis

Kallapur 2013